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The effect of salt concentration on DNA conformation transition: a molecular-dynamics study

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Abstract We performed three 3-ns molecular dynamics simulations of d(CGCGAATTCGCG)$_2$ using the AMBER 8 package to determine the effect of salt concentration on DNA conformational transitions. All the simulations were started with A-DNA, with different salt concentrations, and converged with B-DNA with similar conformational parameters. However, the dynamic processes of the three MD simulations were very different. We found that the conformation transition was slow in the solution with higher salt concentration. To determine the cause of this retardation, we performed three additional 1.5-ns simulations starting with B-DNA and with the salt concentrations corresponding to the simulations mentioned above. However, astonishingly, there was no delayed conformation evolution found in any of the three simulations. Thus, our simulation conclusion is that higher salt concentrations slows the A → B conformation transition, but have no effect on the final stable structure.

Keywords DNA conformation transition · Molecular dynamics simulation · Salt concentration · Sugar pucker · Minor groove width

Introduction

The conformations adopted by DNA and the transition between them are related to biological functions, such as the interactions between DNA and RNA, DNA and protein, etc. The main conformations (A and B forms, shown in Fig. 1) depend on the sequence, ionic environment, and hydration conditions. Ions play an important role in DNA structure by shielding the phosphate charges in the DNA backbone and affecting water activity around DNA [1–5]. Increased salt concentrations favor the formation of A-DNA and Z-DNA over B-DNA [6] and salt effects constitute major electrostatic contributions in the binding of ligands to nucleic acids [7]. A-DNA conformations have been found in solutions with 1 M salt and above [6, 8], whereas in salt concentrations below 1 M, B-DNA is usually prevalent [9, 10].

While diverse experimental biophysical techniques are available for the study of nucleic acid structural properties, no one experimental technique is capable of generating a complete description of the dynamic structure of DNA in its native solution environment. Molecular dynamics (MD) simulation is a technique that can, in principle, provide a complete description of the structures and structural evolution. Since Cheatham and Kollman performed an MD simulation with explicit water and counterions and found the A → B conformation transition of DNA using the AMBER force field in 1996 [11], a number of MD simulations have looked at the nature of A → B transition [12–14]. This transition, which is directly coupled to changes in sugar puckering, has been shown to occur spontaneously in water. However, most of this work concentrated on the average structure analysis or/and were performed only with neutralizing ions. Under such conditions, they found the transition is complete at about 0.5 ns and the final structure is a stable B-like conformation [11]. On the other hand, many MD simulations have been performed to investigate the interaction between ions and DNA, and the ions' distribution around DNA [5, 15–17]. Some added additional salt ions to increase the salt concentration to examine the change of the ion distribution [5].

However, we are interested in how the salt concentration affects the dynamic process of the A → B transition, not only with neutralizing ions, but also with additional ions in solution, and analyzing not only the final stable structure, but also the dynamic process.

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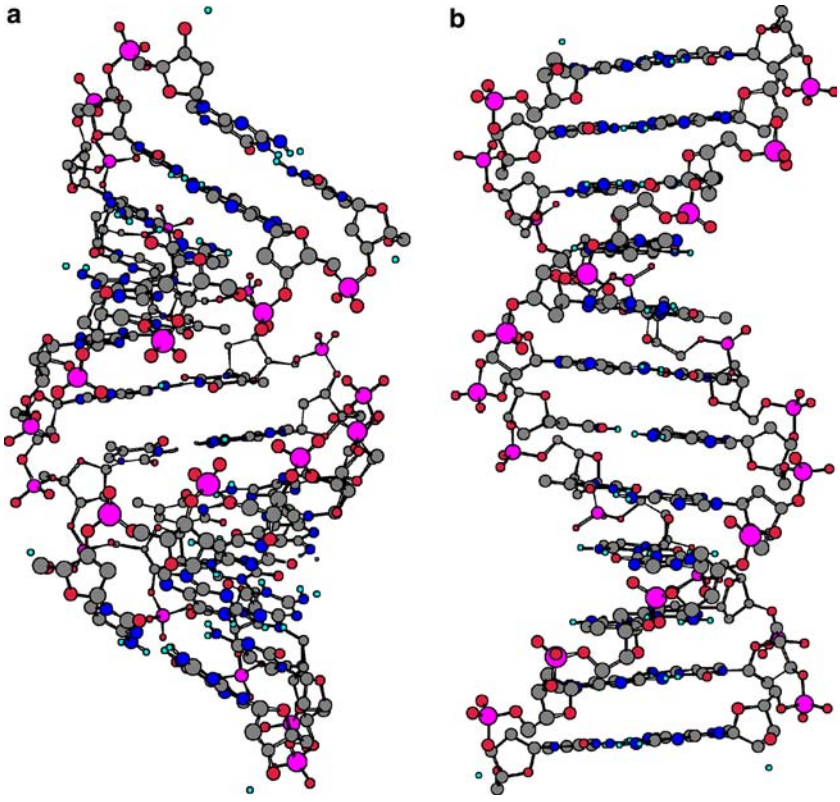

Fig. 1 A-DNA and B-DNA. **a** The canonical A-DNA, and **b** the canonical B-DNA (looking from the central major groove)

We chose the Drew-Dickerson dodecamer d(CGCGAATTCGCG)$_2$ as the simulation object because it has been studied extensively; experimentally and theoretically for its biological relevance. This dodecamer contains the recognition site of the *Eco*RI restriction enzyme and serves as a well-studied reference system.

The most distinctive differences between A- and B-DNA are the minor groove width and sugar pucker. There are several algorithms to calculate these two parameters, but in this study we adopt the 'Curves' algorithm, which has been widely used [18]. For A-DNA, the minor groove width is 11.0 Å or so, and the sugar pucker is 0–36°. For B-DNA the two parameters are about 5.9 Å and 144–180°, respectively. We use these two parameters as the indicators of conformation during the dynamic processes.

Method

First, we performed three MD simulations A1–A3, for a time scale of 3 ns using the AMBER 8 package. The Cornell parm94 force field, which has been shown to be suitable for solvated systems, was used [19]. Every simulation started with the canonical A-form of d(CGCGAATTCGCG)$_2$ generated using the program "nucgen." In simulation A1, we solvated the dodecamer with TIP3P water molecules, resulting in a simulation box of 43.5×43.5×62.3 Å^3, and then 22 Na+ ions were placed by replacing randomly chosen water molecules throughout the simulation box to obtain a neutralized system. In simulation A2, after the above process was finished, 66 additional Na+/Cl− ion pairs were placed in the box, and in simulation A3, 132 additional Na+/Cl− ion pairs were used. These ions were positioned using the program tleap, which is included in the AMBER 8 package. Then simple but effective protocols were adopted: first, 500 steps of minimization were carried out with harmonic restraints of 1500 kcal mol^{-1} Å^2 on the DNA, second, 500 steps of unrestrained minimization were carried out. For the equilibration, first, 10 ps heating up (from 0 to 300 K) and 10 ps equilibrating MD were performed with harmonic restraints of 10 kcal mol^{-1} Å^2 on the DNA using constant-volume and constant-temperature conditions, after which, 100 ps unrestrained equilibration was carried out using constant-pressure and constant-temperature conditions before the trajectory was generated for a further 2880 ps (simulation). The temperature-bath coupling was achieved by the Berendsen algorithm. Long-range interactions were taken into account, via the particle mesh Ewald method (PME). The time step is 2 fs using SHAKE, and snapshots were taken every 1 ps. All the PME and SHAKE parameters were set to default.

After equilibrium was reached, the Na+ ion concentrations of the three simulations were 0.46 M for A1; 1.86 M for A2; 3.27 M for A3.

For data analysis, we used program ptraj to obtain the average structures over the final nanosecond and the root-mean-square deviation (RMSD) over the whole

trajectories of the 3-ns simulations. We also calculated the average minor-groove width and sugar-pucker evolutions versus time using program Curves5.3 [18] combined with our own analysis programs.

The three 1.5-ns additional MD simulations were also performed with the same protocols described above, except that B-DNA was the starting structure, and a similar analysis was performed.

Results

The energy and RMSD of all the atoms in the DNA were used as indicators for judging whether the equilibrium of a system had been reached. The energy of each simulation is stable during the whole production simulation (not shown here), and the RMSDs with respect to the starting structures are shown in Fig. 2. The RMS values indicate that all three simulation systems reach equilibrium within nanosecond scale. The average RMSDs during the last nanoscend for the three simulations were: 4.18 Å for A1; 4.02 Å for A2; 3.66 Å for A3.

Consider the average structures taken from the last nanosecond shown in Fig. 3. The structures are very similar to each other, and the average structure parameters are given in Table 1. From the average snapshots and the parameters, we can see that all three MD simulations give similar average structures, although with different salt concentrations. Actually, the average structures are intermediate states between A- and B-DNA, as others have studied [20, 21], and they are more like B-DNA. This is a deviation from the experiment, which indicates that A-form DNA is favored in the case of high salt concentration [6]. In A2, the salt concentration is about 1.9 M, and in A3, 3.3 M or so. In such salt concentrations, we should have seen some A-DNA-like conformations, but in reality we did not.

The evolutions of the crucial parameters (minor-groove width and sugar pucker) versus time are shown in Fig. 4. We can see obvious A $\rightarrow$ B conformation transitions in all the simulations. At low salt concentration, the transition finishes on about a half-nanosecond scale, which is consistent with the earlier work of Kollman and Cheatham [11]. The two parameters converge to the same values in all three simulations. Furthermore, it is very distinctive that the black line reaches its final stable value first, then the red, and finally the blue. This means that when the salt concentration increases, the A $\rightarrow$ B transition is delayed. This is an interesting result and is discussed in detail below.

Table 1 The minor-groove width and sugar pucker for relaxed canonical A-DNA, canonical B-DNA, and the average structures of the three trajectories

	A-DNA	B-DNA	AVE1	AVE2	AVE3
Width (Å)	10.78	5.72	5.43	5.55	5.91
Pucker (°)	10.57	147.94	126.23	121.85	126.90

The canonical A-DNA and B-DNA were generated using program NUCGEN. The sugar pucker for A-DNA is about 0–36°, and for B-DNA 144–180°

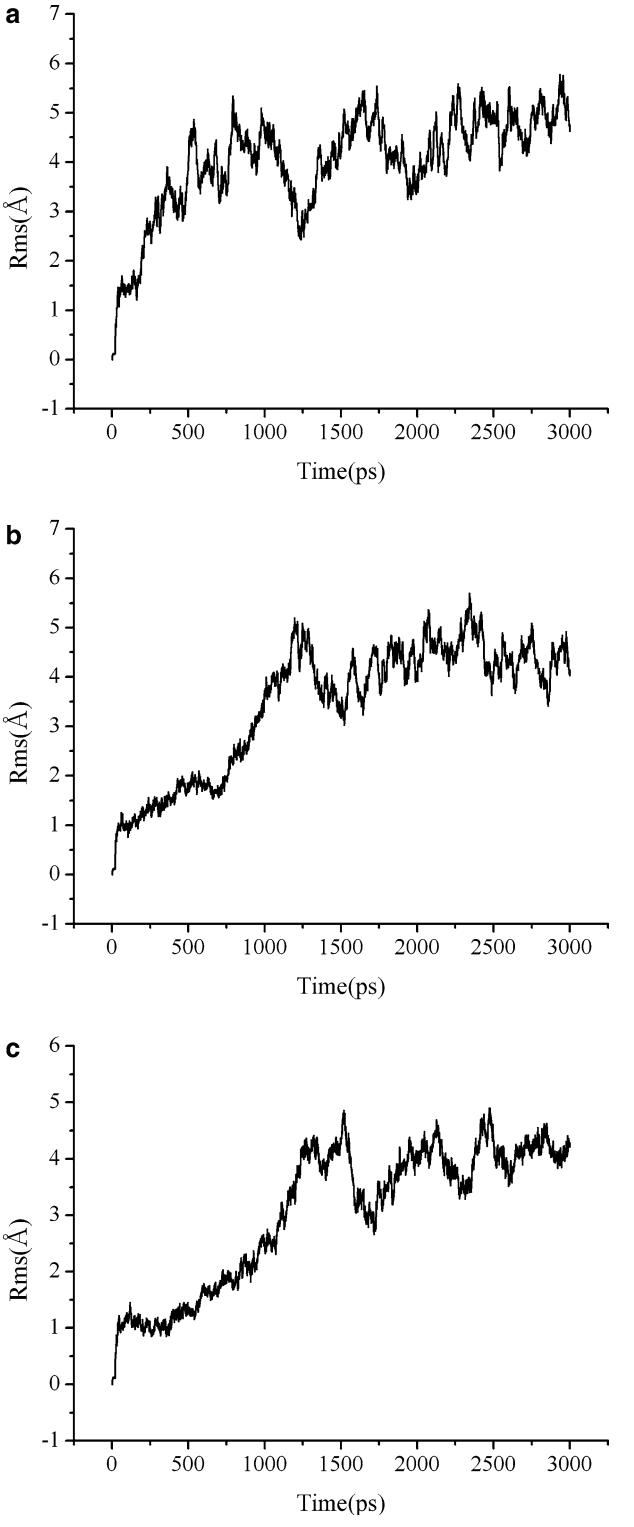

Fig. 2 The RMSDs with respect to the starting structures. **a** For trajectory A1, **b** for A2, and **c** for A3

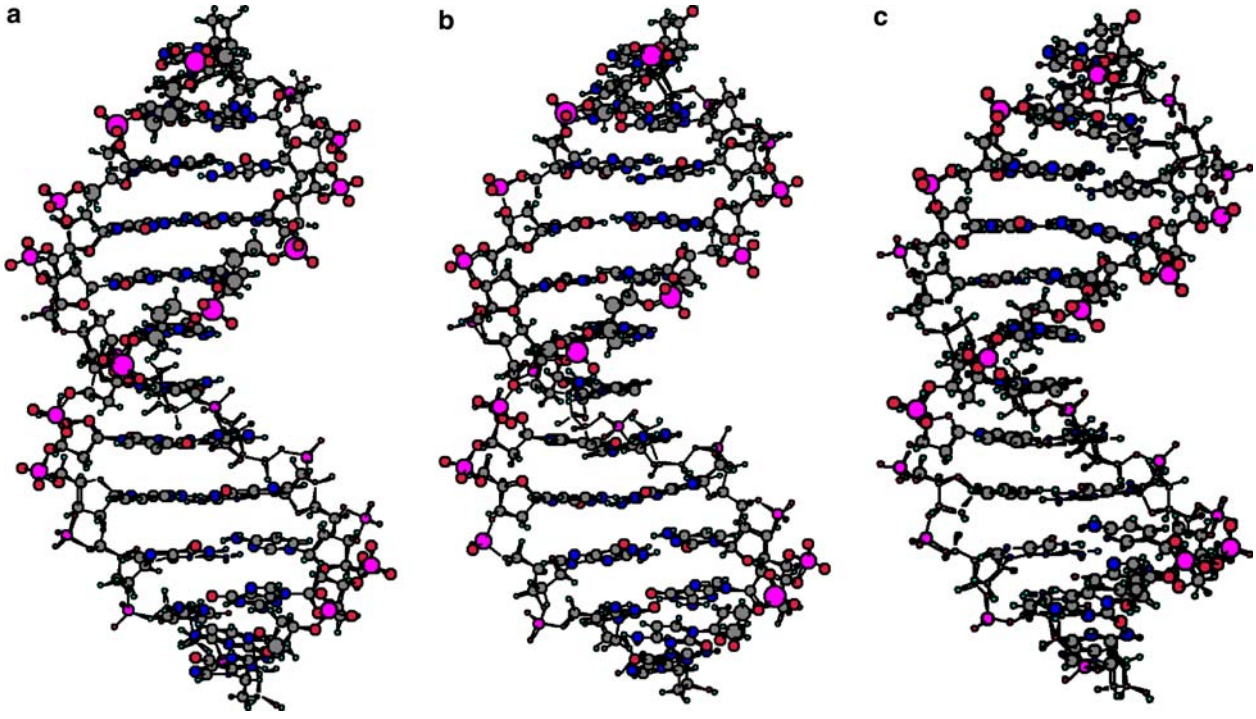

Fig. 3 The average structures taken from the last nanosecond. **a** For A1, **b** for A2, and **c** for A3

Discussion

Two questions now occur. Why do all the simulations with different salt concentrations converge to a similar structure and what causes the delayed A $\rightarrow$ B transition?

For the first question, we think it as an artifact generated by the force field itself. It is well known that different force fields generate different stable structures. An obvious example is that B-DNA is stable in AMBER simulations and A-DNA in CHARMM simulations [20, 21]. The Cornell force field adopted in AMBER may overstabilize the B-DNA. Thus we cannot see the salt concentration effect on the final stable structure. This result is consistent with the earlier work of Kollman and Cheatham, but not consistent with Mazur's simulations, where he found a cooperative and reversible B $\rightarrow$ A transition [12]. However, we note that Mazur performed his simulations in a water drop instead of periodic boundary conditions. As he discussed, one possible reason that resulted in the B $\rightarrow$ A transition was the surface testion, which did not exist in our system with periodic boundary conditions. Thus, the inconsistent results can be explained by the different simulation environments.

There may be two possible answers for the second question. One is that to some extent it is consistent with the experiment, which indicates that A-DNA is stable at higher salt concentration. The A $\rightarrow$ B transition is slowed because of a trend for DNA to stay in the A-form at high salt concentrations. However, because of the overstabilization of B-DNA caused by the Cornell force field, it finally converged to the B form. Another answer is that the additional ions generate some kind of viscosity that slows the conformation transition of DNA in solution and it has nothing to do with the stable A-DNA conformation at higher salt concentration in solution.

To determine what causes the delayed transition at higher salt concentrations, we performed three additional 1.5-ns MD simulations. All the conditions were the same as the earlier three 3-ns simulations except that they started with the canonical B-form DNA. Our idea is that the final stable structures are neither canonical A- nor B-DNA. They are something between A- and B-DNA. If the first answer given above is true, that is, there exists some trend for DNA to stay in the A form at high salt concentration, then the B-starting structure will converge faster at a higher salt concentration because higher salt concentration favors A-DNA over B-DNA. If the second answer is true, that is, the additional ions generate some kind of viscosity that slows the conformation transition of DNA, and then the B-starting structure will also converge slower at higher salt concentration.

However, the result is astonishing. The sugar-pucker evolutions versus time are shown in Fig. 5. We can see that the most important parameter, sugar pucker, has an immediate transition from $\sim$146 to $\sim$128° and then remains stable, independent of the salt concentration. In other words, once we remove the restraint, the sugar-pucker parameter reaches its final stable value. The evolutions of the groove width do not give any valuable

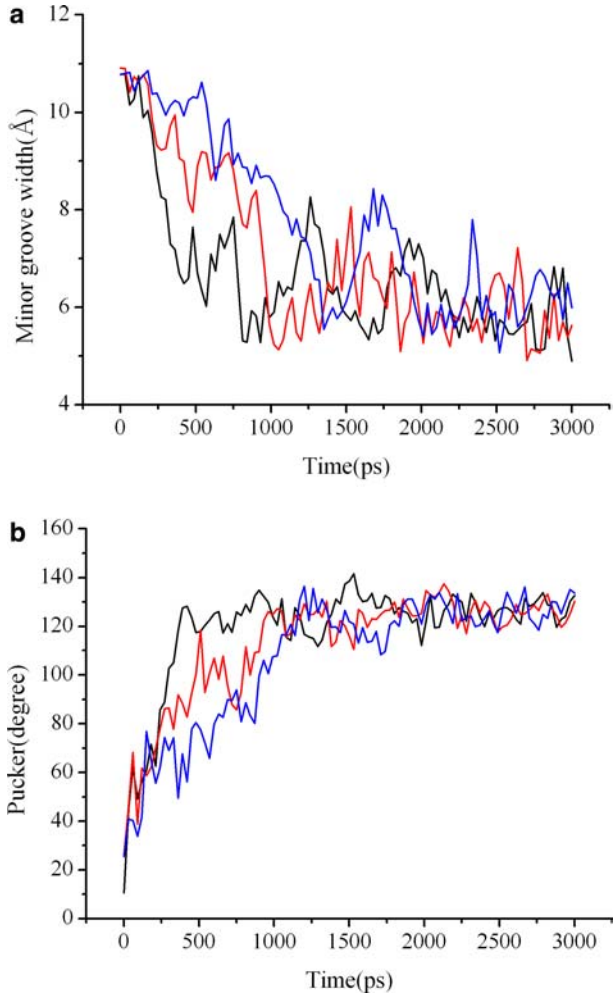

Fig. 4 Minor-groove width and sugar-pucker evolutions versus time. **a** The minor-groove width evolutions versus time, and **b** the sugar-pucker evolutions. Parameter evolutions of simulation A1 are shown in *black*, A2 in *red*, and A3 in *blue*

information, so they are not shown here. This was unexpected. It seems that the final stable structure is much more stable energetically than canonical B-DNA.

For further analysis, we performed a detailed study on the salt distribution around DNA. We found that for the starting A-DNA, many counterions accumulated in the major groove at the first stage of simulation. However, when the simulation time was lengthened, we found that the number of the counterions in the major groove was reduced and the conformation of the DNA gradually changed to the B-form. This is consistent with Mazur's result, which showed fewer counterions in the major groove of B-DNA [12]. We agree with the suggestion of Cheatham and Kollman that the accumulated counterions in the major groove are responsible for the stability of the A-form [22, 23]. Some ions must move out of the major groove simultaneously with the A $\rightarrow$ B transition. We therefore think that when the salt concentration is increased, it takes longer for the counterions to move out of the major groove, and thus the A $\rightarrow$ B transition is slowed. The major-groove-width evolutions versus time were calculated and the results support our idea. The delayed transition at higher salt concentration was seen (as shown in Fig. 6). In fact, the delayed A $\rightarrow$ B transition at higher salt concentration may be caused by complicated interactions between ions and DNA phosphate backbones. However, we still believe that there may be some artifacts in Cornell force field, since we could not reproduce the experimental observations of B $\rightarrow$ A transition in high salt concentration solution. According to the analysis of Cheatham and Young, the ion parameters used may cause underestimation of the interactions between the ions and DNA [24]. Thus, the ion parameters may need to be adjusted first.

From the results and discussion above, we draw the conclusion that, as many people have discovered, AM-

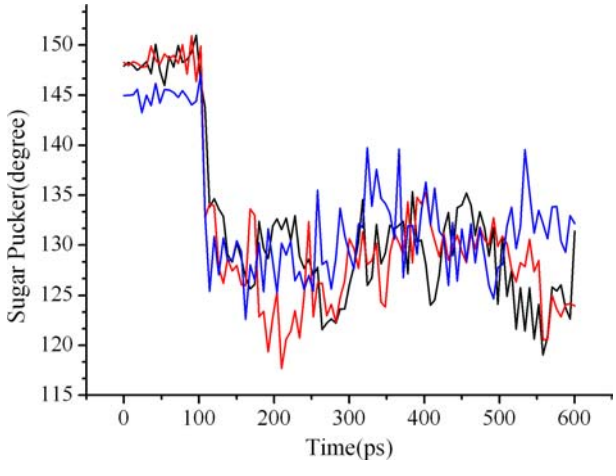

Fig. 5 The sugar-pucker evolutions versus time of the additional simulations starting with canonical B-DNA. The first 600 ps evolutions are shown here

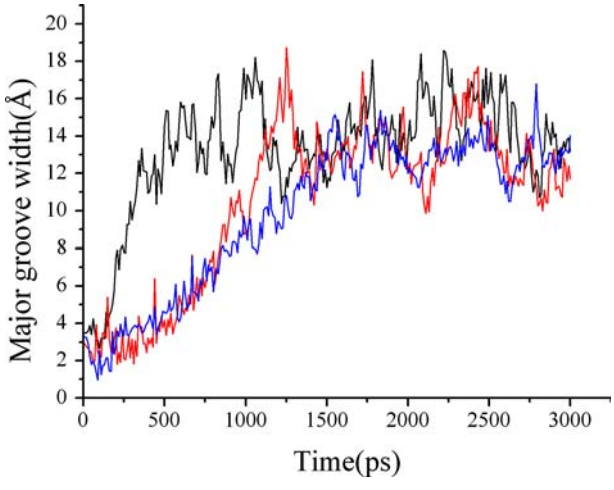

Fig. 6 The major-groove width evolutions versus time. Parameter evolutions of simulation A1 are shown in *black*, A2 in *red*, and A3 in *blue*. We can see similar trends to minor-groove width evolutions, that is, the evolution at higher salt concentration is slower

BER is successful in simulating DNA in solution and has given many meaningful results in DNA studies. We have seen a clear A $\rightarrow$ B conformation transition process and obtained a stable B structure as others have done. We have also seen an obvious delayed A $\rightarrow$ B conformation transition in higher salt concentration solution. However, we think there are still some artifacts in Cornell force field, for example, the overstabilization of B-DNA and misbalance of the ion parameters, leading to the unexpected result that the final stable structures in different salt concentration solutions are all the B-DNA-like conformation. This deviates from the experiment, and it may not be appropriate to simulate DNA evolution in high salt concentration solution using the Cornell force field [19]. Therefore, this work may serve to promote further research to improve the force field used in AMBER.

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